

## Accumulation of Organotin Compounds in Tissues and Organs of Stranded Whales Along the Coasts of Thailand

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**Abstract.** Concentrations of butyltin (BT) and phenyltin (PT) compounds were measured in organs and tissues of five species of whales (Bride's whale [*Balaenoptera edeni*], false killer whale [*Pseudorca crassidens*], pygmy sperm whale [*Kogia breviceps*], short-finned pilot whale [*Globicephala macrohynchus*], and sperm whale [*Physeter macrocephalus*]) found stranded on the coasts of Thailand. The mean concentrations of BTs in various whales were in the range of 0.157 to 1.03 mg kg<sup>-1</sup> wet weight, which were higher levels than the reported concentrations in whales from other countries. PT concentrations were also detected in the range of 0.022 to 1.14 mg kg<sup>-1</sup> wet weight. The concentrations of BTs and PTs in whales were higher than those in mussels from the coastal area of Thailand. Concentrations of tributyltin (TBT) and triphenyltin (TPT) compounds in whale organs and tissues were also compared, and it was found that TBT concentrations were generally higher in liver and lower in lung. TPT concentrations were higher in liver and blubber and lower in lung. Ratios of TBT degradation products in whale liver, namely monobutyltin (MBT) and dibutyltin (DBT), were higher than the ratios of TBT. TPTs in liver were found to be dominant among PTs. The patterns of BTs and PTs in false killer whale liver were different from those in the other whales by cluster analysis. Their concentrations in false killer whales were the highest among all whales in this study. False killer whales feed on squid and large pelagic fish containing higher concentrations of organotin (OT) compounds, so the differences in patterns and concentrations of OTs in liver between false killer whales and the other whales may be caused by difference in diet.

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Organotin (OT) compounds have been used as active biocides in antifouling paints since the early 1960s, and OTs

released into the water column from coatings applied to boat hulls have caused deleterious effects in nontarget marine organisms, such as oysters, bivalves, and gastropods (e.g., Laughlin & Linden 1985; Gibbs & Bryan 1996). Many studies have demonstrated increased concentrations of OTs in the marine environment (e.g., Batley 1989; Short & Sharp 1989; Tolosa *et al.* 1992). Consequently, the serious pollution by antifouling paints containing butyltin (BT) compounds on marine ecosystems has become a great environmental issue worldwide. Some developed countries—such as England, the United States, and France—began regulating the use of OTs as antifouling biocides around 1990. After the implementation of these regulations, the recovery of dogwhelk and oyster populations, as well as a decrease in OT concentrations in the marine environment, were reported in some countries (Evans *et al.* 1995; Ruiz *et al.* 1996). However, higher concentrations of OTs have still been detected in marine mammals. For example, Kannan *et al.* (1997) reported increased accumulation of total BTs of 0.012 to 0.454 mg kg<sup>-1</sup> wet weight in organs of stranded bottlenose dolphins (*Tursiops truncatus*) from along the United States Atlantic and Gulf coasts. BTs in Atlantic spotted dolphins (*Stenella frontalis*) and pygmy sperm whales (*Kogia breviceps*) were also detected at 0.06 to 0.115 and 0.007 to 0.133 mg kg<sup>-1</sup> wet weight, respectively. Tanabe *et al.* (1998) detected BTs in the range of 0.007 to 1.385 mg kg<sup>-1</sup> in cetaceans and seals from North Pacific and Asian coastal waters. It is a serious problem in view of the protection of marine organisms that higher BT concentrations have been detected in marine mammals. Phenyltin (PT) compounds have also been used as antifouling biocides in the countries neighboring Thailand, including Japan. However, few published articles exist on the accumulation of PTs in marine mammals.

OT concentrations were measured in tissues and organs of various whales stranded along the coasts of Thailand, and the current status of OT pollution and body distribution are discussed based on these measurements.

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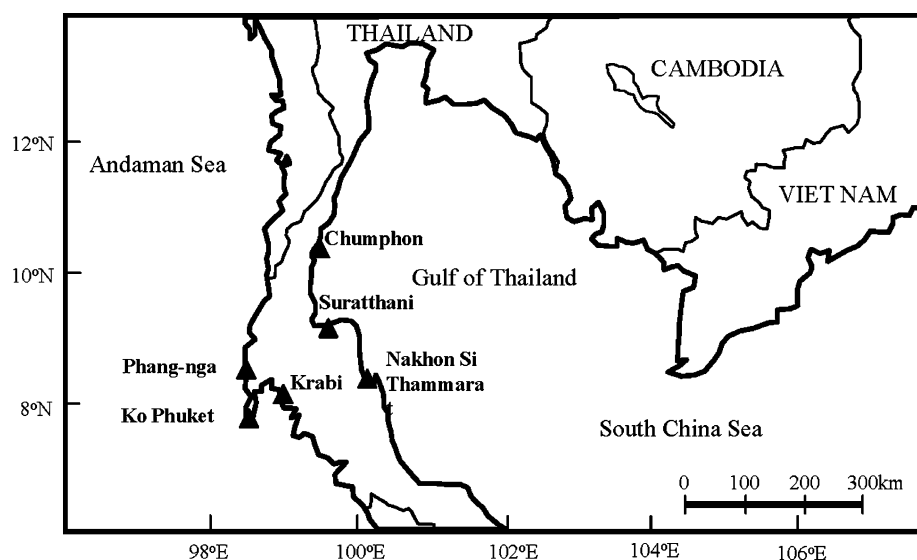


Fig. 1. Sampling locations in Thailand

## Materials and Methods

### Biota Samples

Seven whales from five species—two Bride's whales (*Balaenoptera edeni*), two false killer whales (*Pseudorca crassidens*), one pygmy sperm whale (*Kogia breviceps*), one short-finned pilot whale (*Globicephala macrorhynchus*), and one sperm whale (*Physeter macrocephalus*) found stranded along the coastal of Thailand from 1997 and 2002—were analyzed in this study (Fig. 1). After performing biometric measurements and sex determination (Table 1), the whales were dissected, and tissues and organs were stored in polyethylene bags at  $-25^{\circ}\text{C}$  until analysis. OTs in kidney, muscle, spleen, liver, stomach, heart, blubber, and lung were measured.

### Chemical Analysis

The method used for the determination of BTs and PTs in biologic samples was based on that of Harino *et al.* (2005) with some modification. One gram of the homogenate soft tissues of biologic samples was taken in a centrifuge tube, and 100  $\mu\text{l}$  mixed acetone solution—including 1  $\mu\text{g ml}^{-1}$  each of MBTCl-d<sub>9</sub>, DBTCl-d<sub>18</sub>, TBTCI-d<sub>27</sub>, MPTCl-d<sub>5</sub>, DPTCl-d<sub>10</sub>, and TPTCl-d<sub>15</sub>—was added to the centrifuge tube as surrogate standard. The mixture was extracted with 25 ml 1 M HCl-methanol/ethyl acetate (1/1) by shaking for 10 minutes. After centrifugation for 10 minutes, the supernatant was transferred to the flask. The residue was extracted with 25 ml 1 M HCl-methanol/ethyl acetate (1/1) and centrifuged again. The combined supernatants and 70 ml saturated NaCl solution were transferred to a separatory funnel. The analytes were extracted with 30 ml ethyl acetate/hexane (3/2) solution twice. Then 50 ml hexane was mixed with the combined organic layer, and the mixture was left to stand for 20 minutes. After removal of the aqueous layer, the organic layer was dried with anhydrous  $\text{Na}_2\text{SO}_4$  and was concentrated up to trace level by a rotary evaporator. The analytes were diluted with 5 ml acetic acid–sodium acetate buffer (pH 5.0) and ethylated by the addition of 1 ml 5%  $\text{NaBeT}_4$ . The lipids were saponificated with 10 ml 1 M KOH–ethanol solution by shaking for 1 hour. Next, 40 ml distilled water and 20 ml hexane were added to the solution, and the mixture was shaken for 10 minutes. After removing the organic layer, the analytes were extracted

with 20 ml hexane again. The combined organic layers were dried with anhydrous  $\text{Na}_2\text{SO}_4$ . After concentration up to 1 ml, the solution was cleaned by a Florisil Sep-Pak column (Waters Association). The analytes were eluted with 10 ml 5% diethyl ether/hexane and TeBT-d<sub>36</sub> and TePT-d<sub>20</sub> were then added as internal standards. The final solution was concentrated up to 0.5 ml.

A Hewlett-Packard 6890 series gas chromatograph equipped with a mass spectrometer (5973 N) was used for the analysis of OTs. The separation was conducted by a capillary column coated with 5% phenyl methyl silicone (J & W Scientific; 30 m length x 0.25 mm i.d., 0.25- $\mu\text{m}$  film thickness). The column temperature was held at  $60^{\circ}\text{C}$  for the first 2 minutes, then increased to  $130^{\circ}\text{C}$  at  $20^{\circ}\text{C min}^{-1}$ , to  $210^{\circ}\text{C}$  at  $10^{\circ}\text{C min}^{-1}$ , to  $260^{\circ}\text{C}$  at  $5^{\circ}\text{C min}^{-1}$ , and to  $300^{\circ}\text{C}$  at  $10^{\circ}\text{C min}^{-1}$ . Finally, the column temperature was held at  $300^{\circ}\text{C}$  for 2 minutes. The interface and ion source temperatures and ion energy were  $280^{\circ}\text{C}$ ,  $230^{\circ}\text{C}$ , and 70 eV, respectively. Selected ion monitoring was operated under this programme. Splitless injection (1  $\mu\text{l}$ ) of sample was employed. The concentrations of OTs in this study are expressed as  $\text{Sn}^{4+}$  on a wet-weight basis.

### Data Analysis

The cluster analysis was carried out using STATISTICA. The comparison of OT concentrations was carried out using EXCEL.

## Results and Discussion

### Evaluation of the Analytic Method

To examine the quality of the data obtained by the analytic procedure, 1  $\mu\text{g}$  BT and PT were spiked to 1 g whale liver. The recovery rates and the relative standard deviations (RSDs) of organotin compounds, which were corrected by the deuterated surrogate standards, are listed in Table 2. The recovery rates of BTs and PTs were in the range of 85% to 103% and 87% to 105%, respectively, and the RSDs of BTs and PTs were in the range of 3.2% to 9.2% and 5.1% to 13.2%, respectively. The

**Table 1.** Concentration of OT compounds in tissues and organs of various whales

Common name	Date	Sampling location	Sex	Length (m)	Weight (kg)									
						MBT	DBT	TBT	BTs	MPT	DPT	TPT	PTs	
Bryde's whale	1/12/1999	Phang-nga	Female	4.3	395.71	Blubber	0.093*	0.037	0.083	0.213	0.057	0.013	0.802	0.872
						Heart	0.57	0.021	0.035	0.113	0.055	0.061	0.166	0.237
						Kidney	0.080	0.028	0.054	0.162	0.019	0.013	<0.001	0.032
						Liver	0.063	0.047	0.037	0.147	<0.001	<0.001	0.202	0.202
						Lung	0.098	0.019	0.025	0.142	<0.001	<0.001	0.055	0.055
						Muscle	0.032	0.018	0.028	0.078	0.021	0.007	<0.001	0.028
Bryde's whale	28/6/2002	Suratthani	Male	3.73	-	Blubber	0.062	0.024	0.013	0.099	<0.001	0.014	0.055	0.069
						Heart	0.095	0.015	0.024	0.134	<0.001	0.001	0.029	0.040
						Kidney	0.055	0.039	0.036	0.130	<0.001	0.025	0.081	0.106
						Liver	0.031	0.306	0.071	0.058	<0.001	0.006	0.024	0.030
						Muscle	0.049	0.021	0.028	0.098	<0.001	0.006	0.035	0.041
						Heart	0.049	0.039	0.188	0.276	0.112	0.008	0.007	0.127
False killer whale	24/12/1997	Krabi	Female	3.53	250	Kidney	0.123	0.093	0.192	0.408	0.046	0.010	0.310	0.366
						Liver	0.364	1.647	0.814	2.83	0.237	0.028	0.592	0.857
						Lung	0.040	0.031	0.071	0.142	0.027	0.008	0.008	0.043
						Blubber	0.093	0.053	0.245	0.391	0.067	0.010	0.081	0.158
						Heart	0.064	0.049	0.236	0.349	0.087	0.011	0.165	0.263
						Kidney	0.273	0.129	0.299	0.071	0.096	0.014	0.013	0.1123
False killer whale	26/8/1998	Phuket	Female	3.39	369.5	Liver	0.916	2.870	1.071	4.86	0.549	0.013	0.575	1.14
						Lung	0.636	0.033	0.115	0.784	<0.001	0.014	0.008	0.022
						Muscle	0.066	0.424	0.242	0.732	0.052	0.017	0.694	0.763
						Spleen	0.038	0.032	0.138	0.208	0.011	0.007	0.023	0.041
						Blubber	0.061	0.058	0.022	0.141	<0.001	0.007	0.406	0.413
						Muscle	0.073	0.434	0.140	0.647	0.027	0.005	0.104	0.136
Pygmy killer whale	5/2/2000	Chumphori (with a baby inside, 71 cm in length)	Female	2.6	-	Blubber	0.061	0.058	0.022	0.141	<0.001	0.007	0.406	0.413
						Muscle	0.073	0.434	0.140	0.647	0.027	0.005	0.104	0.136
						Blubber	0.140	0.056	0.127	0.323	0.041	0.017	<0.001	0.058
						Heart	0.042	0.041	0.132	0.215	0.034	0.012	0.015	0.061
						Liver	0.148	0.244	0.154	0.546	0.037	0.013	0.016	0.063
						Muscle	0.063	0.028	0.100	0.191	0.052	0.009	0.006	0.067
Short-finned pilot whale	2/6/2001	Nakhon Si Hammarat	Female	3.38	268	Blubber	0.036	0.018	0.055	0.109	<0.001	0.011	0.402	0.413
						Liver	0.033	0.113	0.139	0.285	0.045	0.011	0.050	0.106
						Muscle	0.033	0.041	0.020	0.094	<0.001	0.008	0.269	0.277

\* Data expressed as mg kg<sup>-1</sup>.

**Table 2.** Recovery rates of organotin compounds in whale liver

Amount (g)	Amount spiked (µg)	Recovery rate (%)					
		MBT	DBT	TBT	MPT	DPT	TPT
1	1	103 (6.9)*	85 (3.2)	97 (9.2)	102 (5.1)	87 (13.2)	105 (11.2)

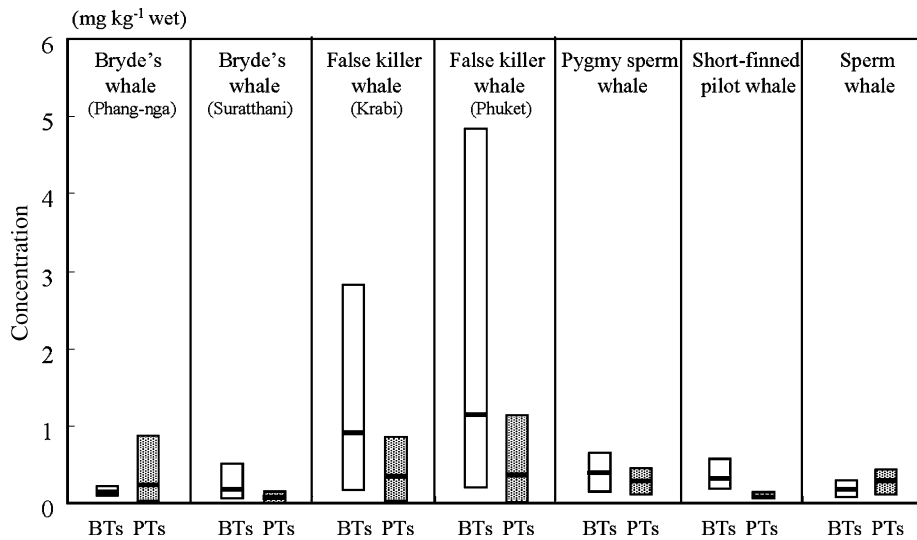
\* Values in parentheses represent the relative standard deviation (n = 4).

detection limits of BTs and PTs, corresponding to a signal-to-noise ratio of three, was 1 µg kg<sup>-1</sup> wet weight for each.

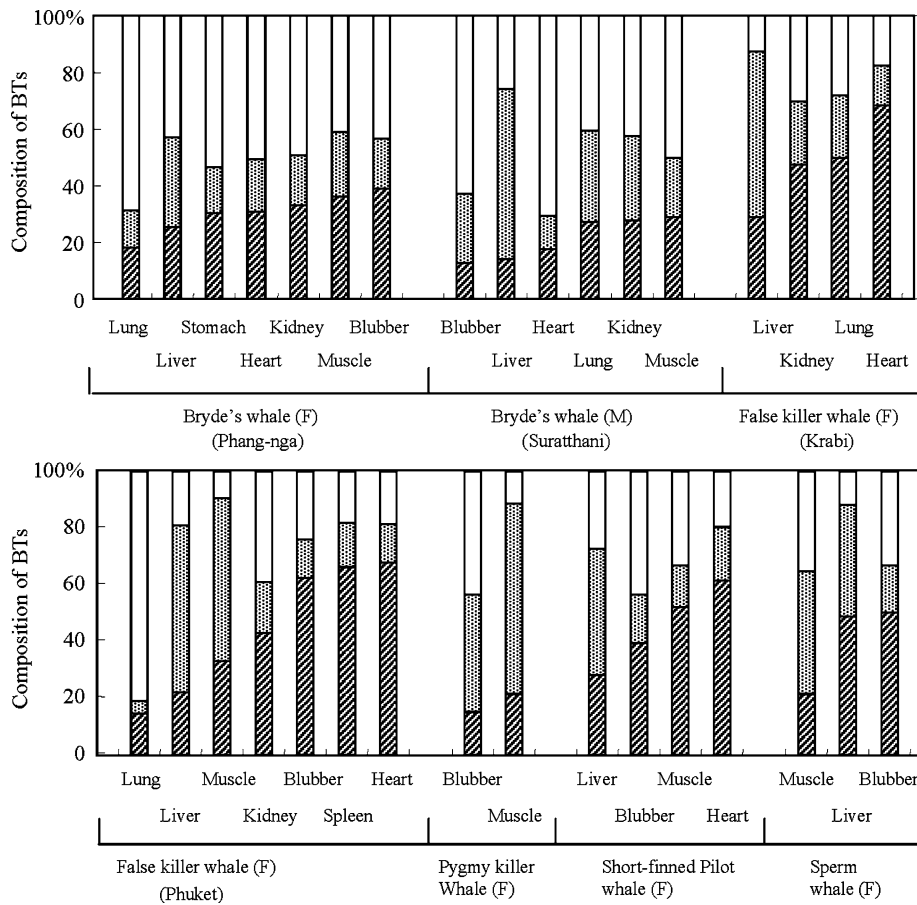
#### Concentrations of OTs in Whales from Thailand

Concentrations of BTs and PTs were measured in organs and tissues of various species of whales from Thailand. The mean concentrations of BTs were 0.157, 1.03, 0.394, 0.318, and 0.163 mg kg<sup>-1</sup> wet weight in organs and tissues of Bryde's whales, false killer whales, one pygmy sperm whale, one

short-finned pilot whale, and one sperm whale, respectively (Fig. 2 and Table 2). Kannan *et al.* (1997) reported the increased accumulation of total BTs (0.012 to 0.454 mg kg<sup>-1</sup> wet weight) in organs of bottlenose dolphins (*Tursiops truncatus*) stranded along the United States Atlantic and Gulf coasts. BTs in Atlantic spotted dolphins and pygmy sperm whales were also detected at 0.06 to 0.115 mg kg<sup>-1</sup> and 0.007 to 0.133 mg kg<sup>-1</sup> wet weight, respectively (Kannan *et al.* 1997). BTs were detected in the range of 0.00099 to 0.1931 mg kg<sup>-1</sup> wet weight in Dall's porpoise (*Phocoenoides dalli*) from Japan (Yang & Miyazaki 2006). BT concentrations in the whales stranded in Thailand were higher compared with other reported values.



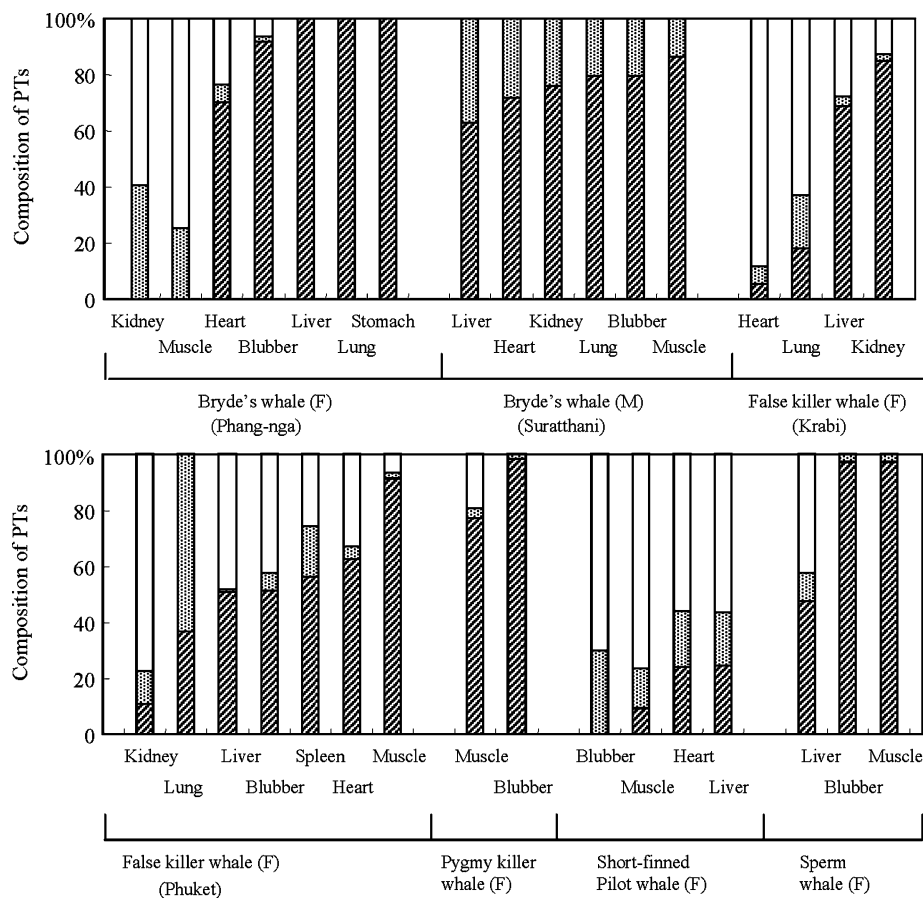
**Fig. 2.** Concentrations of BTs and PTs in various species of whales. Rectangles and horizontal bars represent ranges and means, respectively



**Fig. 3.** Compositions of BTs in tissues and organs of various species of whales. (Hatched bars) TBT. (Dotted bars) DBT. (Open bars) MBT. F = female; M = male

Harino *et al.* (2006) reported BT concentrations in green mussels (*Perna viridis*, *L.*) from the coastal area of Thailand in the range of 0.023 to 0.070 mg kg<sup>-1</sup> wet weight. Namely, BTs in whales were detected at concentrations >10 times greater compared with green mussels. The concentrations of BTs tend to become lower toward the open sea compared with the coastal area because of the low density of ships and well-

flushing water. Nevertheless, OT concentrations in whales in open sea were higher than those in mussels distributed in the coastal area. Bioaccumulation through diet is considered an important factor of OT intake in whales. In fact, the higher concentrations of OTs in fish and squid, which are part of the whale diet, have been reported (Harino *et al.* 2000; Yamada *et al.* 1997). Furthermore, the decrease of P450 content as well



**Fig. 4.** Compositions of PTs in tissues and organs of various species of whales. (Hatched bars) TBT. (Dotted bars) DBT. (Open bars) MBT. F = female; M = male

as the activities of ethoxyresorufin and pentoxyresorufin O-depentylase by TBT in *in vitro* experiments using hepatic microsomes of cetaceans have been reported (Iwata *et al.* 1995; Kim *et al.* 1998). It has been proposed as a hypothesis that higher concentrations of BTs in cetaceans are caused by bioaccumulation by the diet and the decrease of metabolism enzyme for TBT. Further study is needed to clarify the accumulation mechanism of OT compounds in whales.

The mean concentrations of PTs were detected in the ranges of 0.150, 0.353, 0.275, 0.063, and 0.265 mg kg<sup>-1</sup> wet weight in tissues and organs of Bryde's whales, false killer whales, one pygmy sperm whale, one short-finned pilot whale, and one sperm whale, respectively (Fig. 2 and Table 1). Few data exist on PT concentrations in whales. PT concentrations in whales were higher than those in mussels from the coastal area of Thailand (Harino *et al.* 2005). This phenomenon may be caused by bioaccumulation by way of diet and low cytochrome P450 enzyme activities for PTs in whales.

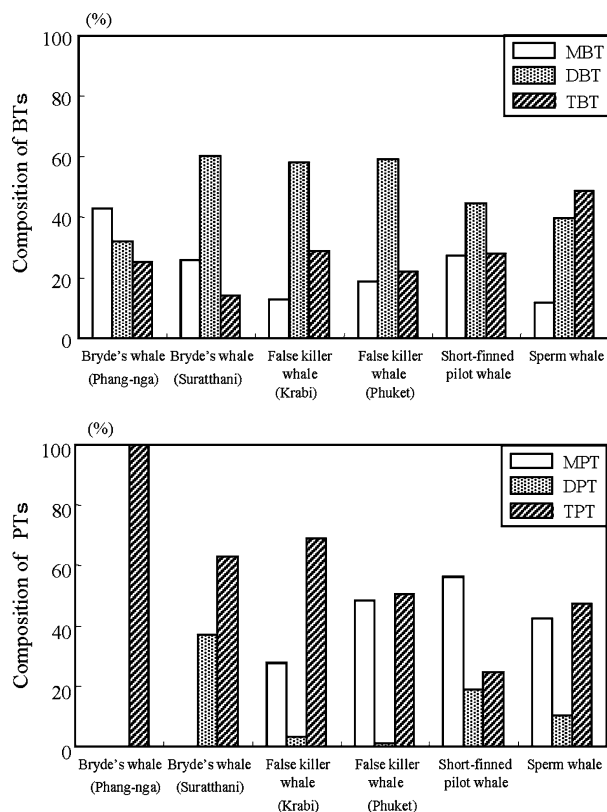
The concentrations of PTs in whales were lower than those of BTs (Fig. 2). Although BTs have been used in antifouling paints worldwide, PTs have not been used as such in most countries. PTs are found in pesticides, but there has generally been little use of PTs compared with BTs. Lower PT concentrations, compared with those of BTs in whales, thus has been attributed to the lower use of PTs.

Concentrations of TBTs in each tissue and organ are listed in Table 1. TBT concentrations in the liver were generally

higher than those found in the other organs. Kannan *et al.* (1997) reported the highest concentrations of BTs in the livers of dolphins and whales stranded along the United States Atlantic and Gulf coasts. Among all tissues and organs of porpoises from Japanese coastal water, the highest BT concentrations were found in liver (Iwata *et al.* 1995). Yang and Miyazaki (2006) also reported the highest concentration of TBT in the livers of Dall's porpoises. Our findings are in agreement with these reported results. In contrast, TBT concentrations in whale lung were low.

TPT concentrations in whale tissues and organs were high in liver (Table 1). Higher concentrations of TPTs were also observed in blubber of Bryde's whales, one pygmy sperm whale, and one sperm whale. It is well known that the hydrophobic compounds, such as polychlorinated biphenyls and DDT, accumulate in blubber in the bodies of striped dolphins (Tanabe *et al.* 1981). It is possible that TPT is more hydrophobic than TBT, judging from the body distribution of TPT. TPT concentration in lung was low, similar to the concentration of TBTs.

The compositions of BTs and PTs in whale tissues and organs are shown in Figures 3 and 4, respectively. The patterns of BTs or PTs varied in tissues and organs. BTs, combined with proteins, are distributed to various tissues and organs by way of blood (Oshima *et al.* 1997; Shimazaki *et al.* 2002). Furthermore, the bioconcentration factor of OTs differs according to the tissues and organs in which they present be-



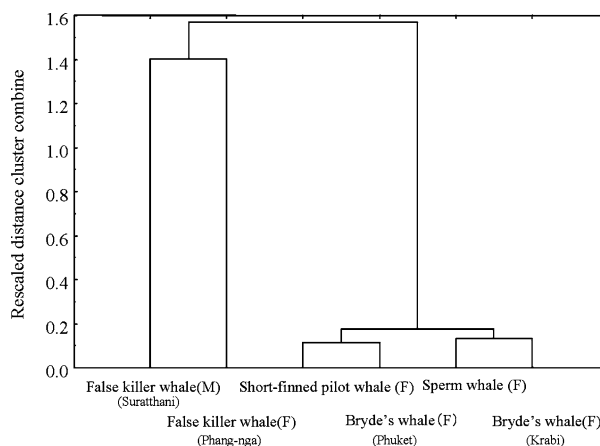
**Fig. 5.** Composition of BTs and PTs in liver of various species of whales

cause of differences in tissue and organ composition and enzyme metabolic activity (Iwata *et al.* 1997). These remarkable differences in patterns of BTs or PTs in tissues and organs may be caused by the differences in various factors, such as the transportation of OTs by blood, the composition of cell membranes, or the metabolism of OTs by enzymes contained in each organ.

#### *BTs and PTs in the Liver of Each Whale*

The compositions of BTs and PTs in liver are shown in Figure 5. The proportion of TBT in total BTs was low in liver, despite the higher BT concentration among tissues and organs. In contrast, TPT was dominant in liver except in the case of the short-finned pilot whale. Harino *et al.* (2000) reported a similar trend in liver of fish. These findings therefore show that TPT in aquatic organisms is more stable compared with TBT.

Cluster analysis was carried out in whales based on the concentrations of each OT in liver (Fig. 6). These whales were divided into two groups, false killer whales and the other whales, based on the composition of BTs or PTs, suggesting differences in metabolism of BTs and PTs in liver between false killer whales and the other whales. BT concentrations in false killer whales were approximately 10 times higher than those in the other whales, and PT concentrations were also higher in false killer whales (Table 1). Iwata *et al.* (1997) suggested bioaccumulation through diet as the cause of increased BT and PT concentrations because the content of the



**Fig. 6.** Cluster analysis based on the concentration of each OT species in liver. F = female; M = male

intestine in one finless porpoise contained a significant levels of BTs. One of the food items of false killer whales is marine mammals, such as dolphins, which accumulate higher concentrations of OTs, in addition to squid and fish (Mark 1996). The higher OT concentration in false killer whales is therefore attributed to the higher trophic level compared with that in the other whales.

#### **Conclusion**

Increased OT concentrations were detected in the organs and tissues of whales, and the OT concentrations in whales was higher than that in mussels from the coastal area of Thailand. Thus, the increased OT concentrations in the top predators of the aquatic ecosystem, such as whales, seem to indicate the transfer of these compounds through the food chain. Further study must be carried out to clarify the transfer process of OTs through the food chain and to evaluate the effect of OTs on whales.

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